

Claims

What is claimed Is:

1. A method of producing embryogenic callus from immature inflorescence explants of St. Augustinegrass plants, comprising:

a) culturing an explant of St. Augustinegrass immature inflorescence to initiate growth of callus tissue; and

b) isolating embryogenic callus which is regenerable into a St. Augustinegrass plant.

2. The method of claim 1, wherein the isolated embryogenic callus is regenerable into an adult St. Augustinegrass plant.

3. The method of claim 1, wherein the isolated embryogenic callus is regenerable into a fertile St. Augustinegrass plant.

4. The method of claim 1, wherein the isolated embryogenic callus is genetically identical to the source from which the explant was obtained.

5. The method of claim 1, wherein the isolated embryogenic callus is regenerable into an adult St. Augustinegrass plant that is genetically identical to the source from which the explant was obtained.

6. The method of claim 1, wherein the immature inflorescence are about 0.5 to about 3.0 centimeters in length at the time of explant harvesting.

7. The method of claim 1, wherein the immature inflorescence-explant is cultured on F1DG callus initiation medium.

8. The method of claim 7, wherein the F1DG callus initiation medium includes proline.

9. The method of claim 1, wherein the steps of culturing the explant to initiate growth of callus and maintaining the embryogenic callus are both carried out in a medium comprising an identical hormone composition.

10. Isolated embryogenic callus produced by the method of claim 3.

11. A method of regenerating St. Augustinegrass plants from immature inflorescence explants, comprising:

a) culturing an explant of St. Augustinegrass immature inflorescences to initiate growth of callus tissue;

b) isolating embryogenic callus from the cultured explant; and

c) regenerating the embryogenic callus into a St. Augustinegrass plant.

12. The method of claim 11, wherein the regenerated St. Augustinegrass plant is an adult plant.

13. The method of claim 11, wherein the regenerated St. Augustinegrass plant is fertile.

14. The method of claim 11, wherein the regenerated St. Augustinegrass plant is genetically identical to the source from which the explant was obtained.

15. The method of claim 11, wherein the immature inflorescence are about 0.5 to about 3.0 centimeters in length at the time of explant harvesting.

16. The method of claim 11, wherein the immature inflorescence explant is cultured in F1DG callus initiation medium.

17. The method of claim 16, wherein the F1DG callus initiation medium includes proline.

18. The method of claim 11, wherein the embryogenic callus is regenerated on SAR regeneration medium.

19. A method of generating transformed St. Augustinegrass plants, comprising:

- a) harvesting St. Augustinegrass immature inflorescences explant tissue;
- b) culturing the explant tissue to initiate callus tissue growth;
- c) isolating embryogenic callus from the explant tissue;
- d) transforming the callus tissue with a DNA vector comprising a transgene(s);
- e) isolating the transformed callus; and
- f) regenerating the transformed callus into a St. Augustinegrass plant.

20. The method of claim 19, wherein the transformed callus is regenerated into mature transgenic St. Augustinegrass plants.

21. The method of claim 19, wherein the transgenic St. Augustinegrass plants can be reproduced by sexual or asexual means.

22. The method of claim 19, wherein the immature inflorescence from which explant tissue is harvested are about 0.5 to about 3.0 centimeters in length at the time of explant harvesting.

23. The method of claim 19, wherein the immature inflorescence explant tissue is cultured on F1DG callus initiation medium.

24. The method of claim 20, wherein the transformed callus is regenerated on SAR regeneration medium.

25. The method of claim 19, wherein the transformed callus is isolated on selection medium comprising a selective agent.

26. A transgenic St. Augustinegrass plant produced by the method of claim 19, the St. Augustinegrass plant comprising a transgene stably integrated into its nuclear genetic material.

27. The transgenic St. Augustinegrass plant of claim 26, wherein the variety of transgenic St. Augustinegrass is selected from the group consisting of Floratine; Bitter Blue; Floratam; Seville; Raleigh; Texas Common; Delmar; Dwarf line 80-10; Dwarf line 6-89-175; Garrets 141; Jade; Woerner's classic; Salzman; Mercedes; Dwarf line 6-89-196; or any combination thereof.

28. The transgenic St. Augustinegrass plant of claim 27, wherein the variety of transgenic St. Augustinegrass is Floratam or Raleigh.

29. The transgenic St. Augustinegrass plant of claim 26, wherein the transgenic St. Augustinegrass plant can transmit the transgene to progeny.

30. The transgenic St. Augustinegrass plant of claim 29, wherein the St. Augustinegrass plant is propagated asexually.

31. The method of claim 19, wherein the transgene conveying improved resistance to an herbicide is selected from the group consisting of: CP4 *epsps*, *bar* and *pat*, or any combination thereof.

32. The method of claim 19, wherein the transgene conveys a phenotype selected from the group consisting of improved resistance to temperature extremes; improved resistance to herbicides; improved resistance to drought; improved shade tolerance; altered plant coloration; altered plant size; improved resistance to fungal, bacterial and viral infection; improved resistance to pests; or any combination thereof.

33. The method of claim 32, wherein the transgene conveys the phenotype of improved resistance to herbicides.

34. The method of claim 32, wherein the transgene conveying improved resistance to temperature extremes is selected from the group consisting of the *E. coli* MnSOD gene; the CAP85 and CAP160 genes of spinach; the soybean SCOF-1 gene; the *Arabidopsis* CBF3 gene; the barley BLT4 gene; the *Arabidopsis* GPAT gene; the *Athrobacter globiformis* gene for choline oxidase (*cod A*); CBF1 and CBF4 genes from *Arabidopsis*; CAT3 gene from maize and CAT1 gene from tomato; DREB1A gene; or any combination thereof.

35. The method of claim 32, wherein the transgene conveying improved resistance to temperature extremes is the CBF1 gene; the CBF4 gene; the DREB1A gene; or the *E. coli* MnSOD gene; or any combination thereof.

36. The method of claim 32, wherein the transgene conveying improved drought resistance is selected from the group consisting of the turgor responsive gene *trg 31*; the bacterial fructan genes; the δ -Pyrroline-5-Carboxylate Synthetase gene; the barley HVA1 gene; the *Arabidopsis* ERD1 gene; the mannitol-1-P dehydrogenase gene; a trehalose synthesis gene; NtC7 gene of tobacco; glutamine synthetase (GS) gene of rice; OsCDPL7 gene of rice; the DRO2 gene; the *E. coli* trehalose synthesis genes (TPSP/TPP); and the DREB2A gene; or any combination thereof.

37. The method of claim 32, wherein the transgene conveying improved drought resistance is the DRO2 gene, the DREB2A gene, a trehalose synthesis gene, or any combination thereof.

38. The method of claim 32, wherein the transgene conveying improved phenotypic characteristics is selected from the group consisting of the 2-oxidase gene; the OsGA20-ox2; the OsGA3-ox2; the BAS1 gene; the *rol* (A, B, C) genes; the *phyA* gene; the *crtO* gene; the lycopene cyclase gene; OsMADS45 gene; and the OsMADS1 gene; or any combination thereof.

39. The method of claim 32, wherein the transgene conveying improved phenotypic characteristics is the 2-oxidase gene or the BAS1 gene, or a combination thereof.

40. The method of claim 32, wherein the transgene conveying improved disease resistance is selected from the group consisting of genes useful in combating southern lawn chinch bug; White grubs; Sod webworms; Armyworms; Cutworms; Fungal infections such as brown patch, gray leaf spot, *Helminthosporium*, *Pythium*, rust and downy mildew; viral infections such as SAD virus; or any combination thereof.

41. The method of claim 32, wherein the transgene conveying improved insect feeding resistance is selected from the group consisting of the *Phaseolus vulgaris* alpha amylase inhibitor; Arcelin 5A seed storage genes; the sweet potato trypsin inhibitor; the *Bacillus thuringiensis* cry1A and cry1B genes; the *Nicotiana glauca* proteinase inhibitor gene; the Mir1 cysteine proteinase inhibitor; the chitinase gene; or any combination thereof.

42. The method of claim 32, wherein the transgenes conveying improved insect feeding resistance are the *Bacillus thuringiensis* cry1A and cry1B genes.

43. The method of claim 32, wherein the transgene conveying improved virus disease resistance is selected from the group consisting of viral coat protein genes; viral NSM genes; viral antisense RNA genes; nuclear inclusion genes; or any combination thereof.

44. The method of claim 32, wherein the transgene conveying improved microbial pathogen disease resistance is selected from the group consisting of beta 1,3-glucanase gene; the cecropin gene; the MeRIP gene of *Mirabilis expansis*; the chitinase gene; antimicrobial peptide genes; or any combination thereof.

45. The method of claim 32, wherein the transgene conveying improved disease resistance is selected from the group consisting of the Rpg1 gene of barley; the NDR1 gene of *Arabidopsis*; various R genes from the superfamilies NB-LRR, eLRR and LRR-kinase; genes that encode transcription factors that regulate genes more directly involved in resistance to diseases organisms; or any combination thereof.

46. The method of claim 32, wherein the transgenes conveying improved disease resistance are various R genes from the superfamilies NB-LRR, eLRR and LRR-kinase or genes that encode transcription factors that regulate genes more directly involved in resistance to diseases organisms, or combinations thereof.

47. The method of claim 19, wherein the transgene is a heterologous promoter.

48. The method of claim 47, wherein the promoter is selected from the group consisting of the cauliflower mosaic virus 35S promoter; the figwort mosaic virus promoter; the maize ubiquitin promoter; the rice actin promoter; the nopaline synthase promoter; or any combination thereof.

49. The method of claim 47, wherein the promoter is selected from the group of promoters controlling the genes consisting of the rd29A gene of *Arabidopsis*; the Pcp1 gene of the ice plant; the blt4 gene of barley; the CPRD genes of cowpea; or any combination thereof.

50. The method of claim 47, wherein the promoter is selected from the group of promoters controlling the genes consisting of the POT9 gene of poplar; the TCH2 gene of *Arabidopsis*; the WAK14 gene of *Arabidopsis*; or any combination thereof.

51. The method of claim 19, wherein the callus tissue is transformed by microprojectile bombardment.

52. The method of claim 19, wherein the callus tissue is transformed by *Agrobacterium*-mediated DNA transfer.

53. The method of claim 19, wherein the callus tissue is transformed by DNA uptake following electroporation.

54. The method of claim 19, wherein the vector comprises a selectable marker and is selected from the group consisting of: pUC19 and pBIN19, derivatives of the same, or any combination thereof.

55. The method of claim 54, wherein the selectable marker is selected from the group consisting of an antibiotic, an enzyme, a herbicide resistance gene, or any combination thereof.

56. The method of claim 54, wherein the selectable marker is selected from the group consisting of CP4, nptII, *bar*, or any combination thereof.

57. The method of claim 25, wherein the selection medium is a hormone-free medium and the selective agent is glyphosate.